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临床研究

骨关节炎软骨细胞对白细胞介素18的炎症应答

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摘要:目的 探讨IL-18对骨关节炎软骨细胞的作用。**方法** 取骨关节炎患者的膝关节软骨,进行原代细胞培养。不同浓度的IL-18(0,50,150,300 ng/mL)刺激软骨细胞,RT-PCR检测TNFα和COX-2 mRNA的表达,ELISA检测TNFα、PGE_2的水平。应用IL-1受体阻断剂(IL-1Ra)+IL-18干预软骨细胞,检测软骨细胞COX-2的表达量和培养液中PGE_2的水平。提取软骨细胞RNA,测定IL-18受体的表达。**结果** 对于COX-2和TNFα,300 ng/mL组和150 ng/mL组mRNA的表达量显著高于对照组($P<0.01$,$P<0.05$)。50、300 ng/mL组的PGE_2水平显著高于对照组($P<0.05$)。150、300 ng/mL组的TNFα蛋白的浓度显著高于对照组($P<0.05$),IL-1Ra组的PGE_2浓度高于对照组($P<0.01$),但是低于IL-18组($P<0.01$)。软骨细胞能够检测到IL-18受体的表达。**结论** IL-18诱导软骨细胞产生炎症应答,这种作用和IL-1β有关但不完全依赖IL-1β。
关键词:骨关节炎;白细胞介素18;软骨细胞;退行性变

Inflammatory responses of human osteoarthritic chondrocytes induced by interleukin 18

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Abstract: Objective To explore the role of interleukin 18(IL-18) on the osteoarthritis (OA) patients' chondrocytes. **Methods** Knee cartilage samples were obtained from osteoarthritic patients, and the primary cells were cultured. After stimulated by IL-18 (0, 50, 150, 300 ng/mL), chondrocytes were collect to obtain the total RNA, and the supernatant were extracted, too. The mRNA' s expression of COX-2 and TNF*α* were detected by quantitative RT-PCR and the level of PGE_2 and TNF*α* were investigated using ELISA. Besides IL-18, IL-1 receptor inhibitor (IL-1Ra) was also added into the medium of cell culture. COX-2 mRNA and PGE_2 were respectively determination. IL-18 receptors in chondrocytes were detected by RT-PCR. **Results** mRNA expression of COX-2 and TNF*α* in 150 and 300 ng/mLwere both significantly higher than that in control group ($P<0.05$). The level of in 50 and 300 ng/mL group were higher than that in control group ($P<0.01$). While the concentration of TNF*α* in 150 and 300 ng/mL groups appeared more than that in control group significantly ($P<0.01$). And the density of PGE_2 in IL-Ra group was significantly more than that in control group, but less than that of IL-18 group. IL-18R can be detected on chondrocytes. **Conclusion** IL-18 induces inflammatory responses in osteoarthritic chondrocytes and that this effect was related with, although not entirely dependent on, IL-1β.
Key words: osteoarthritis; interleukin 18; chondrocytes; degradation

骨性关节炎(OA)是全球最常见的关节疾病,给社会造成巨大的经济负担[1-2]。我国OA的发病率约为4%。60岁以上者膝骨性关节炎发病率高达49%,每年消耗的医疗费用超过1500亿元[3]。因此,早期有效的治疗OA具有重大的社会效益和经济效益。目前OA的治疗方法多样,但是难以阻止病情进展,大量患者最终需接受关节置换手术。虽然手术能够一定程度提高生活质量,但是给患者带来较大的创伤和医疗负担,且存在血栓形成、关节内感染、假体松动等并发症[4-5]。另外,随着人口平均寿命的延长以及OA发病的年轻化,平均寿命为10~15年的关节假体需慎用于65岁以下的患者。因此,如何针对OA的发病机制,寻找新的、有效的治疗方法一直是骨关节炎研究领域的热门课题。

OA是多种炎症因子参与的关节退变疾病。软骨破坏是其主要病理特征,软骨碎片进入滑液,刺激滑膜引起滑膜炎,滑膜释放序贯性的炎性介质,进一步降解软骨,形成恶性循环[6]。白细胞介素1β诱导软骨细胞产生NO、iNOS、PGE_2、MMPs等损伤性的炎症因子[7],导致软骨细胞的炎症反应和软骨基质的降解[8-10]。IL-18的结构与IL-1相似,被认为是IL-1超家族的一员[11-12]。类风湿关节炎(RA)患者的膝关节滑液中可以检测到IL-18的表达,同时,RA的关节滑膜中也可检测到

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IL-18R的存在[13-14]。前人的研究探讨了高浓度的IL-18对RA滑膜细胞和RA患者外周血单核细胞的影响,并发现对后者的促炎作用更加显著。但是,IL-18对OA软骨细胞的作用尚未有确切报道。IL-1能够诱导PGE_2的产生和COX-2的基因表达,并诱导iNOS的产生[15]。但是,IL-1β和IL-18在这种作用中的关系尚不清楚。本研究探讨了IL-18在OA发病过程中的作用,分析了IL-18和IL-1在促炎作用中的关系,为OA的靶向治疗提供理论依据。

1 材料与方法

1.1 细胞培养与分组

实验所用的软骨细胞来自8名OA患者(5女,3男,平均年龄62.3岁)。取材过程获得患者的知情同意。关节软骨切成2~3 mm^3的小块,DMEM(Gibco,USA)冲洗3次,10%的胰蛋白酶37 ℃下消化15 min。收集组织,加入0.2%的II型胶原酶(Gibco, USA)和0.1%的透明质酸酶(Sigma, USA)37 ℃摇床消化6 h。除去消化酶,DMEM清洗,无菌细胞筛过滤。用含10% FBS的DMEM悬浮细胞并进行原代培养。倒置相差显微镜观察软骨细胞的形态。检测软骨细胞的特异性胶原——Ⅱ型胶原进行细胞鉴定。原代细胞接种至六孔板。原代培养的软骨细胞加入不同浓度的IL-18(0、50、150和300 ng/mL)孵育48 h,收集上清液,完成ELISA检查。提取细胞总RNA,进行后续PCR实验。

为进一步研究IL-18和IL-1的炎症诱导作用,取5例软骨细胞分为3组:对照组不加干预,IL-18组加入100 ng/mL的IL-18,IL-1Ra(IL-1受体抑制剂)组软骨细胞加入100 ng/mL的IL-18和IL-1Ra(R&D,USA),孵育24 h。收集细胞上清液,ELISA法检测PGE_2的水平。

1.2 细胞免疫化学检测

8 mm×8 mm载玻片泡酸并消毒后放入六孔板,接种软骨细胞,培养24 h,完成爬片。弃去培养基,PBS清洗3次,每次3 min。3%双氧水封闭内源性过氧化物酶,动物血清封闭非特异性蛋白。去除残留血清,加入II型胶原兔多克隆抗体(Boster,China),放入湿盒中4 ℃过夜。加入羊多克隆抗体(Boster,China),37 ℃孵育30 min。加入辣根过氧化物酶底物和DAB显色剂,倒置显微镜下观察并采集图像。

1.3 细胞因子检测

收集细胞培养上清液至1.5 mL离心管,3000 g/min离心后放−80 ℃低温冰箱保存,集中测定细胞因子浓度。应用酶联免疫吸附试验(ELISA),根据试剂盒厂家的说明书(PGE_2: Uscn Life, China; TNFα: Cusabio, China)绘制标准曲线,计算PGE_2和TNFα的浓度。

1.4 RT-PCR检测

应用Trizol(Invitrogen, USA)裂解培养的软骨细胞,提取总RNA。260 nm分光光度计(NanoDrop, Wilmington, USA)测定mRNA浓度。应用逆转录试剂盒(Toyobo, Japan)(Applied Biosystems, Foster City, CA)进行逆转录反应(Applied biosystems, Foster City, CA)。反应条件:30 ℃-10 min, 42 ℃-20 min, 95℃ -5 min。应用ABI7500 PCR(Applied biosystems, Foster City, CA) 系统完成定量PCR。双delta Ct法进行定量分析[16]。取PCR产物进行凝胶电泳分析。取4例患者软骨细胞,参考Moller等的研究检测IL-18Rα和IL-18Rβ的表达[17]。

1.5 统计分析

应用SPSS13.0软件包进行统计分析。细胞因子的浓度及mRNA水平均表示为均数±标准差,$P<0.05$有统计学差异。不同浓度IL-18对软骨细的胞影响采用多样本比较的方差分析,若有统计学差异,则采用LSD法或者Dunnetts'T_3(方差不齐时)进行多重比较。

2 结果

2.1 软骨细胞的形态观察和鉴定

接种后1 d软骨细胞呈圆形或椭圆形,透光度高(图1A),细胞稳定贴壁后成多边形,核大(图1B)。软骨细胞Ⅱ型胶原染色阳性,细胞间分布有网状的Ⅱ型胶原(图1C),对照组未见Ⅱ型胶原阳性染色(图1D)。

2.2 RT-PCR和ELISA检测炎症因子的水平

随着IL-18浓度的升高,COX-2和TNFα的mRNA表达量升高(图2)。无论是COX-2还是TNFα,300 ng/mL组和150 ng/mL组的表达量显著高于对照组($P<0.01$, $P<0.05$)。检测不同浓度IL-18干预下,炎症蛋白的水平(表1)。50 ng/mL组、150 ng/mL组和300 ng/mL的PGE_2浓度分别为410.03±22.97、460.38±112.86、518.28±38.80 pg/mL,均高于对照组(340.47±22.03 pg/mL,$P=0.003$,$P<0.001$和$P<0.001$,图3A)。同样,与对照组相比,150 ng/mL组和300 ng/mL组的TNFα(52.45±10.93 pg/mL和103.80±22.29 pg/mL)显著增高($P=0.002$,$P<0.001$,图3B)。IL-18诱导PGE_2和TNFα的增高,呈浓度依赖效应。

2.3 IL-1Ra对软骨细胞炎症应答的影响

IL-18干预下,无论是否加入IL-1Ra,COX-2的表达上调(图4)。对照组、IL-18组和IL-1Ra组的PGE_2浓度分别为140.26±23.63 pg/mL、399.27±70.27 pg/mL和268.16±36.45 pg/mL, IL-18组和IL-Ra组PGE2的水平显著高于对照组($P<0.001$,$P=0.002$),而IL-18组的PGE_2浓度显著高于IL-Ra对照组($P=0.003$,图4)。

2.4 RT-PCR检测IL-18R的表达

RT-PCT检查软骨细胞IL-18受体的表达(图5)。尽管表达强度不一致,软骨细胞中均可检测到IL-18Rα的表达,2例检测到IL-18β的表达。图中呈现的是4例

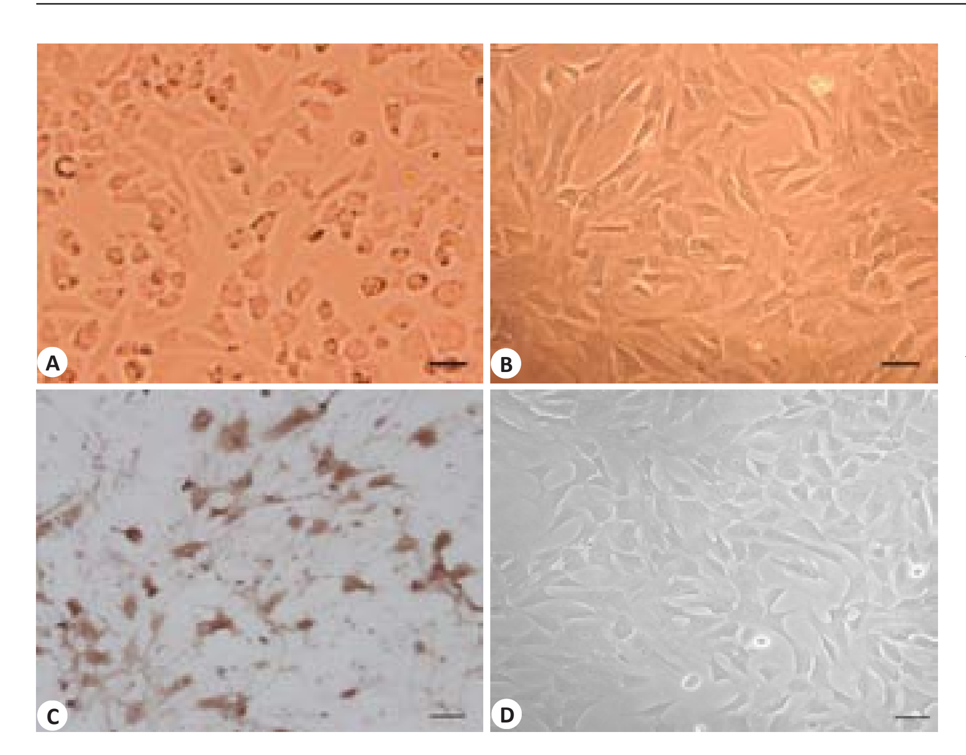


图1 软骨细胞的形态图
A: 显示细胞接种24 h, 部分细胞贴壁,未贴壁细胞呈球形; *B*: 显示典型软骨细胞呈多边形; *C*: 软骨细胞鉴定,棕色区域为Ⅱ型胶原阳性染色, 分布于胞浆和细胞间; *D*: 对照组, 无阳性染色. 标记线为100 μm.

表1 IL-18干预后PGE_2、TNF-α的水平($\bar{x}\pm s$, pg/mL)

Group	PGE_2 (n=8)	TNF-α (n=8)
0 ng/mL	340.47±22.03	20.48±7.36
50 ng/mL	410.03±22.97*	25.32±9.55
150 ng/mL	460.38±112.86*	52.45±10.93#
300 ng/mL	518.28±38.80*	103.80±22.29#

*P<0.01 *vs* PGE_2 in 0 ng/mL group; #P<0.01 *vs* TNF-α in 0 ng/mL group.

患者软骨细胞(B)中IL-18Rα和IL-18β mRNA的表达。

3 讨论

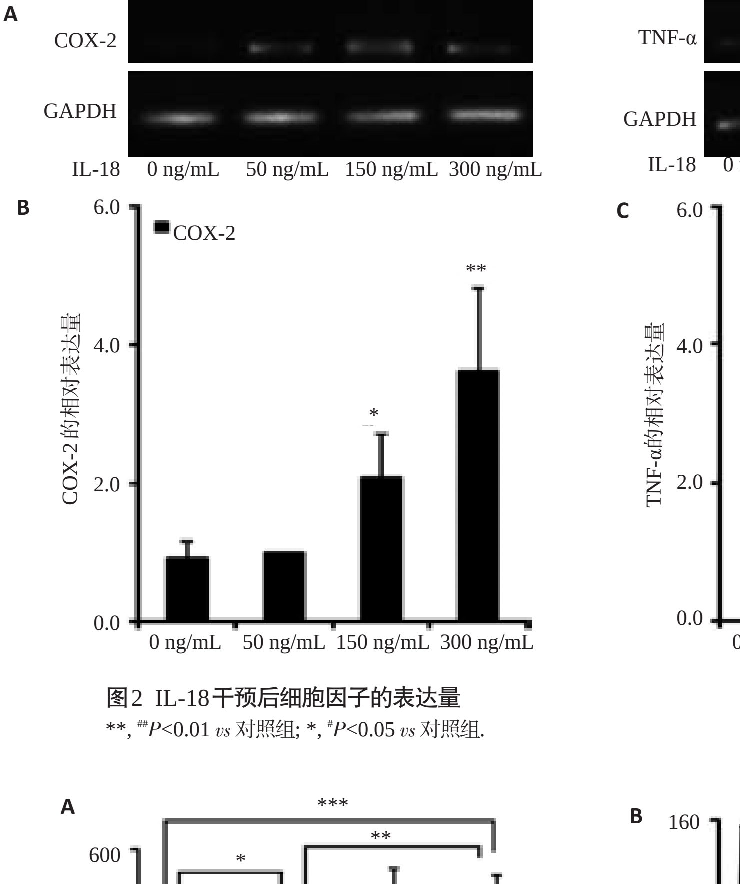
IL-18诱导OA软骨细胞的炎症应答。本研究发现IL-18能够诱导软骨细胞表达TNFα和PGE_2,刺激软骨细胞生成过量的COX-2。COX-2通过降解花生四烯酸产生PGE_2。一旦PGE_2过量,将导致软骨代谢失衡,诱发软骨降解[18]。关节液中TNFα的水平和OA病情紧密紧密相关,是OA炎症进展的促进因素[19-20]。另外,本研究还发现OA软骨细胞均表达IL-18Rα,且部分表达IL-18Rβ。IL-18对细胞的刺激依赖于这两种受体,然后通过NF-κB途径完成信号转导,引起细胞炎症应答[22]。

IL-18可能通过上调某些细胞因子的含量,引起OA的症状。TNF-α是OA病理过程中重要的细胞因子之一,许多研究发现OA的功能评分与TNF-α的含量具有相关性[19, 21]。另外,TNF-α和PGE_2一起加重OA患者膝关节的疼痛、肿胀等临床症状。Notoya等[23]也对IL-18和TNF-α做了部分研究,并发现,由于IL-18能够诱导IL-1β和TNF-α的mRNA表达,后两者在对软骨细胞的作用类似于IL-18。IL-18对软骨细胞的影响可能依赖于IL-1或TNFα,或者受到二者的影响[23]。

IL-18和IL-1β对软骨细胞的作用既有交叉又相互独立。IL-1β促进软骨细胞分泌炎症因子,并分泌基质金属蛋白酶,导致软骨基质的降解,是OA发病过程中典型的促炎因子[24-25]。IL-18在功能上与IL-1β有相似之处,二者均促进软骨细胞分泌炎症因子。然而,IL-1Ra阻断IL-1β的作用后,IL-18仍然能成功诱导软骨细胞的炎症应答,但是PGE_2和TNFα的水平有所降低。这表明IL-18发挥作用部分依赖于IL-1β,但并不全依赖于IL-1β。可能与二者的传导通路有关。IL-18通过MAPK-p38-AP1途径和NF-κB途径促进COX-2的产生[26-27],而IL-1β可以通过p38 APK/c-Fos/AP-1和JAK2/STAT1/2途径诱导基质金属蛋白酶的产生。不同的信号通路导致了IL-18和IL-1β的不同效应。

关节液主要来自滑膜细胞的分泌和软骨细胞的部分代谢产物,是关节软骨自我更新所依赖的环节。OA患者滑膜细胞产生更多的IL-18,并通过类似自分泌的方式影响滑膜细胞[28],总体结果增加了关节液中IL-18的浓度。我们的体外实验一定程度上模拟了软骨细胞在高浓度IL-18的干预下主要促炎因子的变化,体外特定的干预有利于剔除活体代谢中的混杂因素。但是,由于正常情况下关节液较少,且为伦理学所限制,目前尚无大样本的研究确定生理状态、病理状态下关节液中IL-18的浓度。我们体外实验中干预浓度的确定一方面参考了前人的研究[29],另一方面是基于预实验的结果。尽管如此,仍能说明IL-18对软骨炎症代谢活动的影响。

IL-18能够增加典型炎症因子的表达,可能处于炎



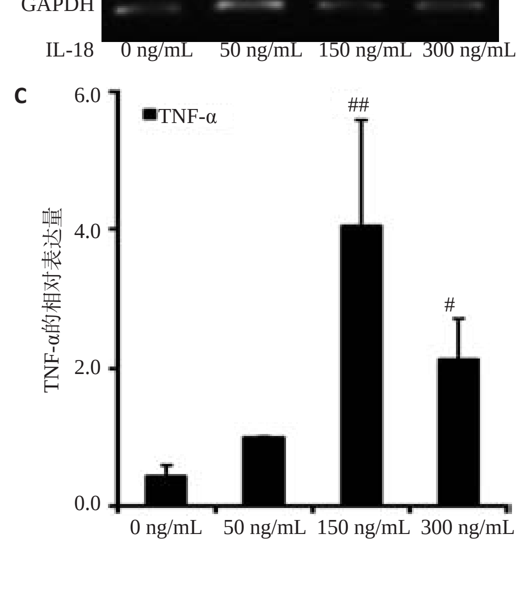
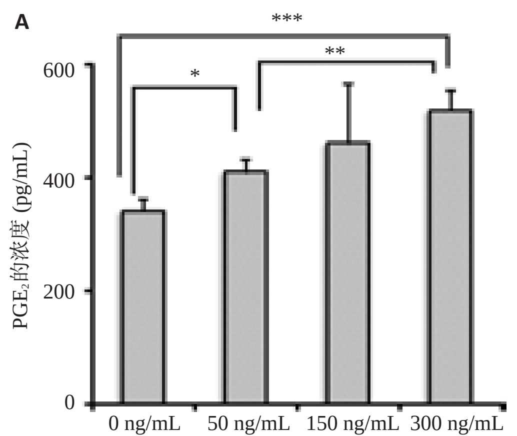


图2 IL-18干预后细胞因子的表达量

**, ##P<0.01 *vs* 对照组; *, #P<0.05 *vs* 对照组.



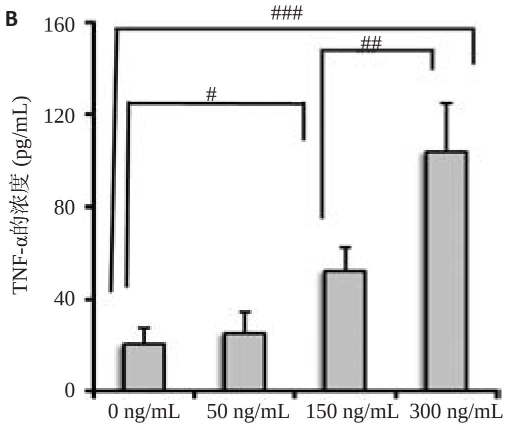


图3 IL-18干预下炎症蛋白的浓度

*, ##P=0.001; **P=0.001; ***P=0.001; #P<0.001; ###P<0.001.

性反应的上游。既往的研究表明,IL-1在OA的发病过程中起重要的作用,本实验发现IL-18对OA软骨细胞的影响可能与IL-1有关,但是其促炎作用相对独立于IL-1对软骨细胞的影响。因此,阻断或抑制IL-18的代谢过程可能对实现早期OA的靶向治疗具有重要的价值。

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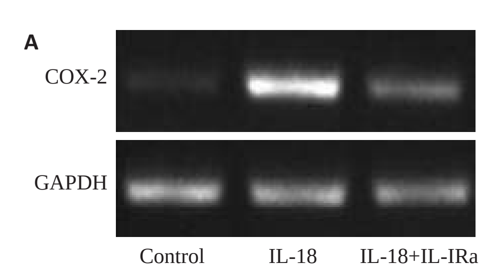
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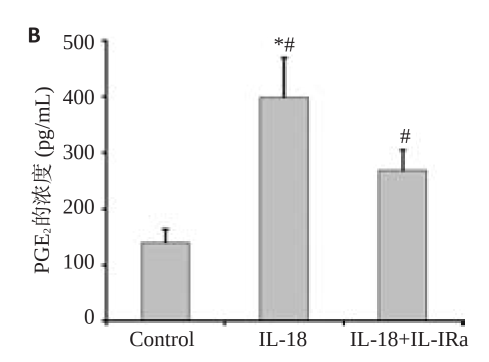


图4 IL-18和IL-Ra干预下炎症因子的表达

#*P*<0.01 *vs*对照组; **P*<0.01 *vs* IL-18+IL-1Ra组.

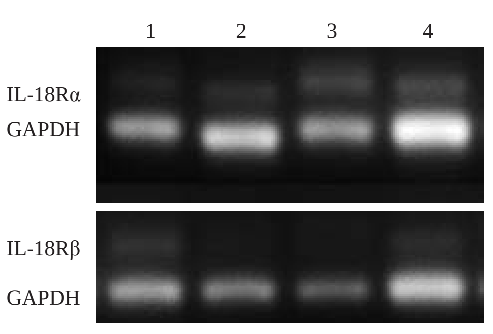


图5 RT-PCR检测IL-18受体的表达

in osteoarthritic chondrocytes and effects of fluid-induced shear on this expression in normal human chondrocytes *in vitro*[J]. J Orthop Res, 1996, 14(1): 67-73.

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